Clinical Imaging of Head and Neck Cancer with Technetium-99m-Labeled Monoclonal Antibody E48 IgG or F(ab')$_2$

Remco de Bree, Jan C. Roos, Jasper J. Quak, Wim den Hollander, Michiel W.M. van den Brekel, Jacqueline E. van der Wal, Hilde Tobi, Gordon B. Snow and Guus A.M.S. van Dongen

Departments of Otolaryngology/Head and Neck Surgery, Nuclear Medicine and Oral Pathology, Free University Hospital, Amsterdam, The Netherlands

Methods: In 32 patients who were suspected of having a neck lymph node metastasis from a histologically proven squamous-cell carcinoma of the head and neck (HNSCC), the diagnostic value of ^{99m}Tc-labeled (750 MBq) monoclonal antibody (1–2 mg) E48 IgG (n = 17) and its F(ab')$_2$ fragment (n = 15) was evaluated and compared. Preoperative findings on lymph node status obtained by radioimmunoscintigraphy (RIS), computerized tomography (CT), magnetic resonance imaging (MRI) and palpation were defined per side (left and/or right side of the neck) as well as per lymph node level (I through V) and compared to the histopathological outcome of the neck dissection specimen. **Results:** All 31 tumors at the primary site were visualized. RIS was correct in 201 of 221 levels (accuracy 91%) and in 38 of 47 sides (accuracy 81%). Fifteen levels and seven sides with limited tumor load were scored false-negative and five levels and two sides were scored false-positive. Sensitivity and specificity of RIS were similar to those of palpation, CT and MRI. The diagnostic value of RIS with E48 F(ab')$_2$ or E48 IgG appeared to be similar. **Conclusions:** The present study shows that RIS with either E48 F(ab')$_2$ or E48 IgG is as valuable as the other imaging techniques. The selective accumulation of radioactivity in tumor tissues, in combination with the known intrinsic radiosensitivity of HNSCC, justifies the development of radioimmunoconjugates for radioimmunotherapy.

Key Words: radioimmunoscintigraphy; monoclonal antibody E48; head and neck cancer; squamous-cell carcinoma

J Nucl Med 1994; 35:775–783

Squamous-cell carcinoma (SCC) is the major histological type of neoplasm arising from the head and neck. Since the status of the cervical lymph nodes is the single most important tumor-related prognostic factor, it is of great importance to know the exact involvement of the neck nodes (*1,2,3*).

The assessment of the status of the neck nodes is mainly based on palpation, although this is generally accepted to be inaccurate. The overall error in the assessment of the presence or absence of cervical lymph node metastasis is 20%–30% (*4*). The high false-positive and false-negative rates of palpation cause over- and undertreatment in many patients. Modern imaging techniques like computerized tomography (CT), magnetic resonance imaging (MRI) and ultrasound (US) appear to be superior to palpation. Since none of these techniques is fully accurate, the need for a more reliable technique remains. US-guided aspiration cytology has been proven to be superior to the current imaging techniques (*5*). However, this technique strongly depends on the skill of the ultrasonographer and cytopathologist. Therefore, the assessment of the status of the neck in patients with SCC of the head and neck (HNSCC) needs further improvement.

Radioimmunoscintigraphy (RIS) with monoclonal antibodies (Mabs) is an innovative imaging technique (*6*). In our institute we developed a murine Mab, designated E48, with high specificity to HNSCC (*7*). A strong reactivity of Mab E48 was seen toward primary as well as metastatic HNSCC (*8*). The antibody has been demonstrated to localizes HNSCC in a nude mouse model with high specificity (*9*). In this murine model, E48 F(ab')$_2$ fragment was shown to be superior for tumor detection as compared to the intact (IgG) antibody (*10*). Therefore, we preferred to use ^{99m}Tc-labeled E48 F(ab')$_2$ in the first clinical study to assess the safety and accuracy of RIS with this Mab in patients with histologically proven HNSCC and clinically suspected for having lymph node metastases.

Recently, promising preliminary results in the first 10 HNSCC patients were published (*11*). RIS was found to detect all primary tumors and to be correct in 13 of 13 tumor-involved neck sides and 17 of 20 tumor-involved lymph node levels. Results with RIS were slightly better than with palpation, CT or MRI. An unexpected finding in this study was the consistent uptake of activity in the normal oral mucosa and the adrenals. Uptake in the mouth may hamper the diagnosis of oral cancer and submandibular and subdigastric lymph nodes in RIS. Uptake in the adrenal glands may be a problem

Received Aug. 25, 1993; revision accepted Jan. 20, 1994.

For correspondence and reprints contact: R. de Bree, MD, Dept. of Otolaryngology/Head and Neck Surgery, Free University Hospital, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands.

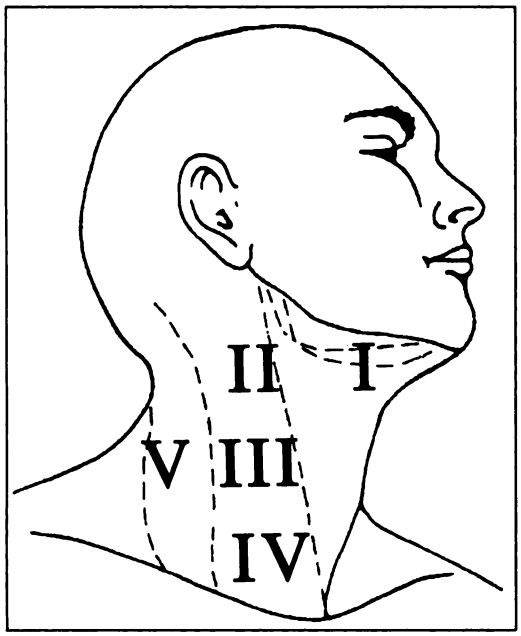

FIGURE 1. Lymph node levels in the neck according to the Memorial Sloan-Kettering Cancer Center Classification: I = submandibular; II = subdigastric; III = midjugular; IV = lowjugular; and V = posterior cervical triangle.

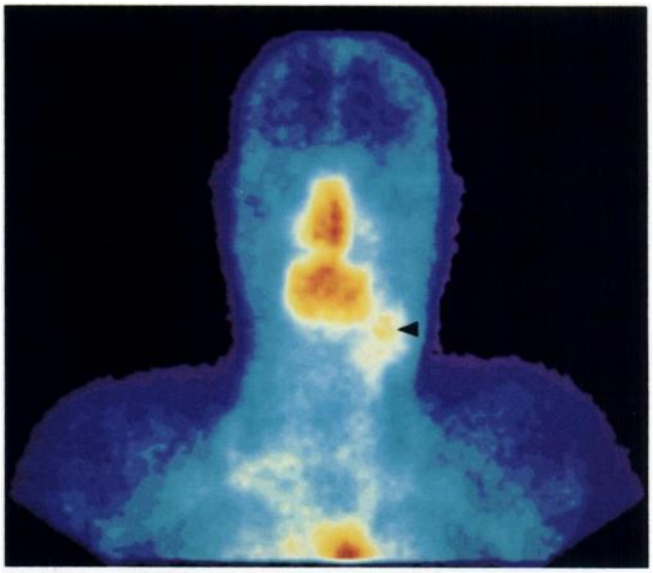

FIGURE 2. Patient 1. Planar anterior image of the head and neck 21 hr after injection of ^{99m}Tc-labeled E48 F(ab')$_2$. Note the intense activity in the nose and mouth. The primary tumor in the left tonsil is not visible on this planar anterior image, probably due to high activity in the mouth. Uptake is seen in the left subdigastric and midjugular lymph node metastases (arrowhead).

in radioimmunotherapy (RIT). Until now, no explanation has been found for the adrenal uptake. Immunohistochemical evaluation prior to this study showed no reactivity of Mab E48 with frozen adrenal tissues. The uptake in the mouth is probably due to specific antigen-antibody interaction, in which the immunoconjugate has overcome the natural barriers offered by the capillary endothelium and the basement membrane of the mucosal epithelium. We hypothesized that the uptake in normal mucosa of the oral cavity might be reduced when using ^{99m}Tc-labeled E48 IgG.

Based on the first promising results (*11*), we extended the RIS study with the option in mind to use either Mab E48 F(ab')$_2$ or IgG in future RIT studies. In the present report, imaging results with E48 F(ab')$_2$ in 15 HNSCC patients are compared to those of E48 IgG in 17 patients.

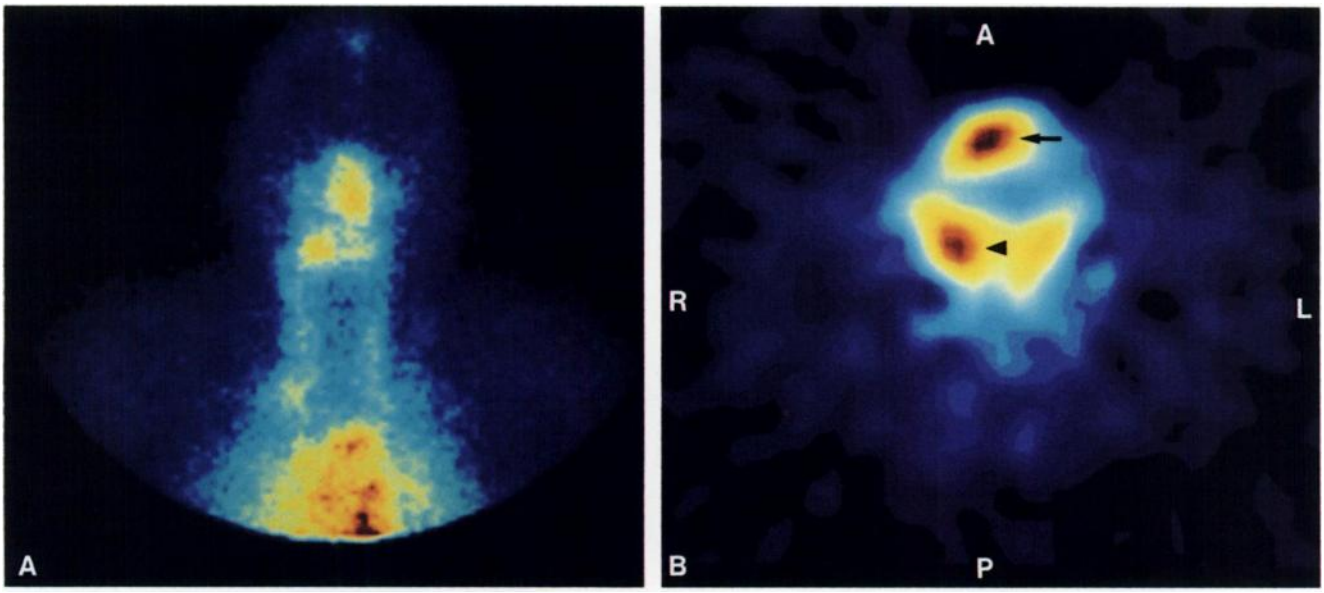

FIGURE 3. (A) Patient 32. Planar anterior image of the head and neck 21 hr after administration of ^{99m}Tc-labeled E48 IgG. Increased activity is seen in the mouth region on the right. Only one spot can be distinguished. (B) Axial SPECT image of the same patient shows two separate spots representing the primary tumor in the anterior floor of mouth (arrow) and the subdigastric lymph node metastases (arrowhead) on the right. A = anterior; P = posterior; L = left; R = right.

PATIENTS AND METHODS

Patient Studies

The protocol was approved by the Dutch Health Council and by the institutional review board of the Free University Hospital. Informed consent was obtained from all participants.

Thirty-two patients, who were at risk for having neck lymph node metastasis from a histologically proven HNSCC and planned to undergo neck dissection(s), participated in this study. The primary tumor and the status of neck lymph nodes were classified according to the TNM system of the International Union Against Cancer, the UICC (*12*). One patient had his primary tumor resected previously. Prior to enrollment, a biopsy of the primary tumor, if available, had to show a positive immunoperoxidase staining with Mab E48. Before and up to 7 days after administration of ^{99m}Tc-labeled Mab E48, urine and blood were obtained for chemical analysis and assessment of Mab pharmacokinetics. Electrolytes, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, lactate dehydrogenase, urea nitrogen, creatinine and uric acid were determined in serum. Hematological determinations included hemoglobin, hematocrit, platelet count, white blood cell count and differentiation and sedimentation rate. Skin tests were not performed. Vital signs were recorded before and up to 3 hr after injection.

Fifteen patients received 1–2 mg of E48 F(ab')$_2$ radiolabeled with ^{99m}Tc (mean dose: 780 MBq, range 560–895 MBq) by intravenous injection over 5 min. Two of them received 10 mg of unlabeled E48 F(ab')$_2$ 1 hr prior to the administration of the labeled Mab with the option in mind to block the adrenal uptake. Seventeen patients received 1–2 mg of E48 IgG radiolabeled with ^{99m}Tc (mean dose 760 MBq, range 510–865 MBq) by intravenous injection over 5 min. Patients receiving E48 F(ab')$_2$, respectively E48 IgG, suffered from carcinoma of the larynx (n = 5, respectively n = 5), tonsil (n = 2, n = 4), oral cavity except tongue (n = 4, n = 5), tongue (n = 2, n = 3), nose (n = 1, n = 0) and lower lip (n = 1, n = 0). The age of the patients receiving E48 F(ab')$_2$ was 61.7 ± 7.8 yr (mean ± s.d.) and for those receiving E48 IgG 57.9 ± 9.1 yr.

Monoclonal Antibody E48

Mab E48 was derived from mice immunized with cells from a metastasis of a moderately differentiated SCC of the larynx ($T_3N_1M^+$). The antigen recognized by Mab E48 was found to be expressed by 94% of the primary head and neck tumors (n = 128) and by the majority of cells within these tumors. A comparable reactivity pattern was observed in 26 tumor-infiltrated lymph nodes from neck dissection specimens (*8*). Antibody reactivity with normal tissue is restricted to normal stratified squamous epithelium and urothelium of the bladder.

Antibody Preparation

The E48 IgG and F(ab')$_2$ used in this study were supplied by Centocor Inc. (Leiden, the Netherlands). E48 IgG was purified from a concentrated tissue culture supernatant by affinity chromatography on a protein A-Sepharose column. For virus inactivation, IgG from the protein A eluate was treated for at least 6 hr with Tween 80 and tri-*n*-butylphosphate. The protein A-purified IgG was further purified on Q-Sepharose and subsequently digested to F(ab')$_2$ by pepsin at pH 3.9. The F(ab')$_2$ fragments were further purified by protein A chromatography to remove residual undigested IgG, followed by elution over a S-Sepharose column. The purity of F(ab')$_2$ preparations was evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under nonreducing conditions and appeared to be more than 95%. This product was filtered through a 0.2-μm filter and dispensed aseptically in a closed environment under anaerobic conditions. The preparation was found to be pyrogenic free.

Preparation of ^{99m}Tc-Labeled E48 IgG and F(ab')$_2$

All radiolabeling procedures were performed under aseptic conditions in a shielded laminar flow hood. All glassware, plastics and solutions were sterile and pyrogen free. For labeling Mab E48 IgG or F(ab')$_2$ with ^{99m}Tc, a modification of the multistep procedure as described by Fritzberg et al. (*13*) was followed, using a S-benzoylmercaptoglycylglycylglycine chelator which was a gift of Mallinckrodt Medical B.V. (Petten, the Netherlands). The purified E48 IgG and F(ab')$_2$ were labeled with a specific activity of 566 ± 172 MBq/mg and 629 ± 166 MBq/mg protein, respectively. A mean of 98.2% ± 1.1% and 98.1% ± 0.9% of the ^{99m}Tc was bound to IgG and F(ab')$_2$, respectively as determined by chromatography on ITLC-SG strips (Gelman Sciences, Ann Arbor, MI) with 0.1 *M* citrate buffer, pH 5.0. Every radiolabeled E48 IgG and E48 F(ab')$_2$ preparation was assayed for immunoreactivity by measuring the binding to gluteraldehyde fixed cells of the vulva SCC cell line A431 (*10*). As determined by a modified Lineweaver-Burk plot, the immunoreactive fractions of ^{99m}Tc-labeled E48 IgG and F(ab')$_2$ at infinite antigen excess were 80.6% ± 14.3% and 77.0% ± 6.7%, respectively. The affinity constants were $1.5 \times 10^{10}\ M^{-1}$ for E48 IgG and $1.2 \times 10^{10}\ M^{-1}$ for F(ab')$_2$ as determined by the Scatchard plot.

Imaging Studies

All patients were examined by palpation, CT, MRI and RIS of the neck prior to surgery. Preoperative palpation was performed by the same experienced head and neck surgeon. CT scans were obtained with a third generation Philips Tomoscan 350 (Philips Medical Systems, Best, the Netherlands) or with a fourth generation Siemens Somaton Plus (Siemens AG, Erlangen, Germany) after intravenous administration of contrast medium (Ultravist 300 mg iodine/ml, Schering AG, Germany). Contiguous axial 5–6-mm scanning planes were used. MRI examinations were done on a 0.6 Tesla imaging system (Teslacon, Technicare, General Electric, Milwaukee, WI) using a partial volume coil. Axial T1-weighted spin echo and Gadolinium-diethylenetriaminepentaacetic acid (Magnevist, Schering AG, Germany) enhanced T1-weighted gradient recalled echo images were made in all patients without claustrophobia. Slice thickness varied from 3 to 5 mm, with an interslice gap of 50% as described by Van den Brekel et al. (*14*). Criteria for the optimal assessment of cervical lymph node metastases by CT or MRI, as defined in our institute, were used. With CT and MRI, neck levels were considered malignant if nodes with necrosis were depicted or if the minimal diameter in the axial plane of a node was 11 mm or more for nodes located in Level II (subdigastric) and 10 mm or more for all other nodes, or if groups of three or more borderline lymph nodes (1 or 2 mm smaller) were seen (*15*).

The radioimmunoscintigrams were obtained with a large field-of-view gamma camera (Gemini, General Electric, Milwaukee, WI) equipped with a low-energy, parallel-hole collimator, connected to a computer (Bartec, Farnborough, United Kingdom). Whole-body images (anterior and posterior views) and planar images of the head and neck (anterior views) were obtained immediately, 16 hr and 21 hr after injection. SPECT images of the head and neck were acquired 16 hr after injection, while lateral scans of the head and neck were obtained 21 hr after injection.

Planar images included the following acquisition parameters: matrix size 128 × 128 (head and neck) or 256 × 256 (whole body) and at least 100 kilocounts were obtained per view during 5–20 min. Acquisition data for SPECT imaging: 64 angles were recorded; 30-sec acquisition per angle; 360° circular orbit; and matrix size 64 × 64. Interpretation of increased uptake of activity was based on asymmetry and retention, especially on late images.

CT, MRI or RIS examinations were each scored by an experienced examiner. All examiners were blinded to the results of other examinations and the pathological outcome. They were only informed about the site of the primary tumor. All patients had uni- or bilateral neck dissections performed between 2 and 7 days after administration of the radioimmunoconjugate. After fixation, all palpable and visible lymph nodes were dissected from the surgical specimen and cut into 2–4-mm thick slices for microscopic examination. The size of lymph nodes does not change by fixation (*15*). The different slices of one lymph node were examined by a pathologist and the percentage of tumor involvement was estimated. The outcome of the histopathological examination of the neck dissection specimens was used as the gold standard.

For topographical evaluation, the findings were recorded per side as well as per lymph node level (Fig. 1) according to the Memorial Sloan-Kettering Cancer Center Classification (*16*). Level I includes the contents of the submental and the submandibular triangles. Levels II, III and IV include the lymph nodes adjacent to the internal jugular vein and the lymph nodes contained within the fibroadipose tissue located medial to the sternocleidomastoid muscle. This area is arbitrarily divided into three equal parts, Level II being the highest and Level IV the lowest level. Level V includes the contents of the posterior cervical triangle. Lymph nodes located outside these levels are mentioned separately. These lymph nodes are included in the evaluation per side, but are not included in the evaluation per level.

Statistical Analysis

Statistical analysis was focused on the comparison of diagnostic tests. As usual, comparison of the quality of diagnostic tests was based on sensitivity, specificity and accuracy. Since these parameters were proportions, confidence intervals could be constructed in two ways. If the denominator was smaller than 100, exact 95% confidence intervals were determined. In larger samples, confidence intervals were calculated using the normal approximation. To assess agreement between tests, the measured kappa (κ) was used (*17*) which reflects agreement between tests beyond the agreement expected by chance alone. The size of κ depends both on the diagnostic tests and the group of patients. When κ is systematically smaller for one group of patients than for another, it is likely that this group is more difficult to diagnose than the other group.

RESULTS

No adverse reactions were observed which could be related to the injection of the antibody and no significant changes were noted in the blood and urine analysis. One patient developed exanthema which was probably related to a recently started diuretic treatment.

Aside from all 30 histopathologically confirmed primary tumors, one occult larynx carcinoma was visualized. Whole-body images up to 21 hr-postinjection showed blood- pool activity with visualization of liver, lungs, heart, spleen, kidneys and nose. Blood-pool activity 16 and 21 hr postinjection was probably less for E48 $F(ab')_2$ than for E48 IgG (Figs. 2, 3A, 4A and 4B) as can be explained by the faster clearance of $F(ab')_2$ from the blood: $F(ab')_2$ $t_{1/2}\alpha$: 2.23 ± 0.41 hr, $t_{1/2}\beta$: 19.6 ± 4.46 hr and IgG $t_{1/2}\alpha$: 6.61 ± 0.59 hr and $t_{1/2}\beta$: 78.10 ± 15.01 hr. For average blood disappearance curves of E48 $F(ab')_2$ and E48 IgG see Figure 5. Uptake of activity was also seen in the adrenals, scrotal area, gallbladder and sometimes intestine, at 16 and 21 hr postinjection as described previously (*11*), and appeared to be similar for $F(ab')_2$ and IgG. Besides this, uptake in the mouth was observed which seemed to be lower for E48 IgG in comparison to E48 $F(ab')_2$ (see Figs. 2 and 3A for representative anterior images). After injection of 10 mg of unlabeled E48 $F(ab')_2$ 1 hr prior to administration of labeled E48 $F(ab')_2$, the adrenals showed lower uptake. In one of these two patients, no adrenals were visualized.

Thirty-two patients underwent a neck dissection with 15 receiving a bilateral neck dissection. A total of 47 neck dissection specimens containing 221 levels were histopathologically examined. Forty-eight levels in 32 sides from 25 patients contained metastases of HNSCC. In one patient the quality of the MRI examination did not allow reliable interpretation because of movement artifacts. Four patients refused MRI examination because of claustrophobia. These patients were not included in the evaluation of the MRI examination. Due to technical problems with the camera system, no SPECT images were acquired in four patients receiving E48 $F(ab')_2$. In one patient containing an undifferentiated small cell tumor with locally squamous cell differentiation, Level III on the left side proved to contain a metastasis of an undifferentiated small cell carcinoma. Because no HNSCC metastasis was found, this lymph node was not included in the analysis.

The findings on palpation, CT, MRI and RIS were analyzed for each level and side and correlated with histopathological examinations (Tables 1 and 2, respectively). Sensitivity of RIS, palpation, CT and MRI for the whole group of patients was 68%, 64%, 67% and 62% per level and 79%, 88%, 88% and 83% per side, respectively. Only 7 of the 32 levels, which were scored true-positive by RIS, were clearly visualized on planar images. In all other cases SPECT images provided additional information (Figs. 3 and 4). Specificity of RIS, palpation, CT and MRI was 97%, 95%, 95% and 97% per level and 86%, 79%, 79% and 70% per side, respectively. Interpretation of RIS for the whole group was correct in 201 of 221 levels (accuracy 91%) and 38 of 47 sides (accuracy 81%). RIS findings were false-positive in five levels and two sides. Fifteen levels and seven sides were scored false-negative. Accuracy of palpation, CT and MRI was 88%, 89% and 89% per level, respectively, and 85%, 85% and 79% per side, respectively.

Sensitivity per level was 83% for E48 $F(ab')_2$ and 54% for E48 IgG. Sensitivity per side for these groups was, 94% and 65%, respectively. For E48 $F(ab')_2$ and E48 IgG, the specificity of RIS was 99% and 95% per level and 100% and

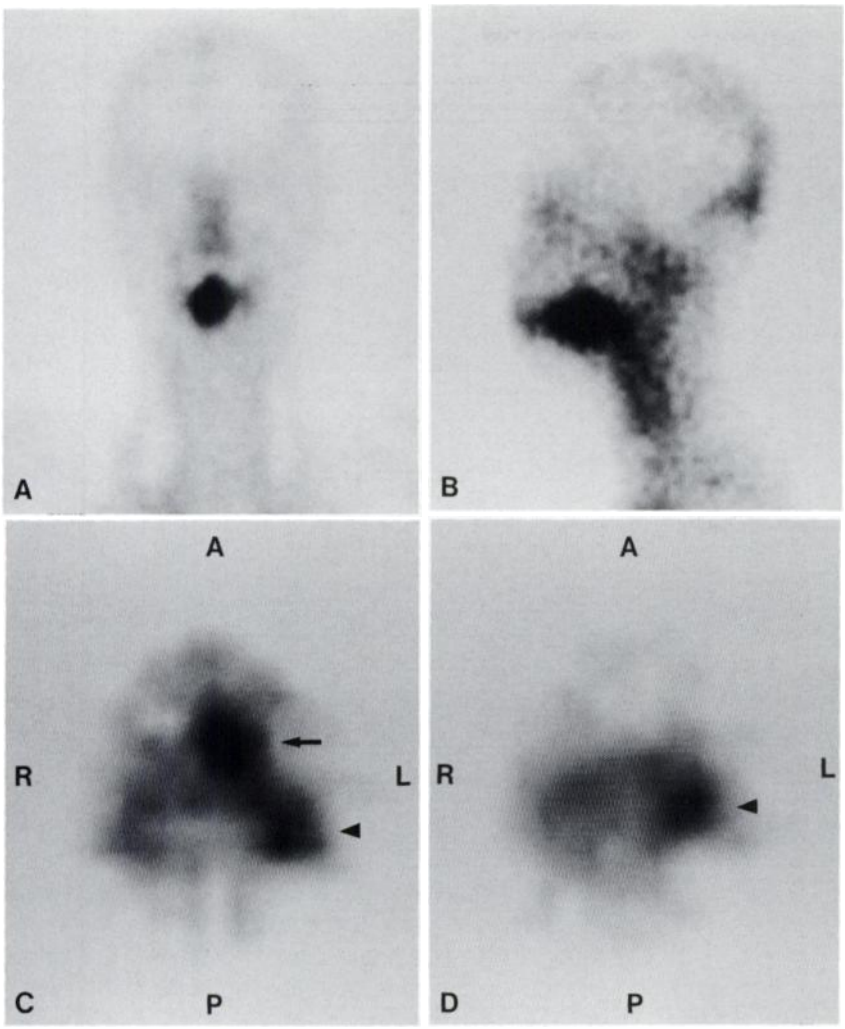

FIGURE 4. Patient 23. (A) Planar anterior image of the head and neck 21 hr after injection of ^{99m}Tc-labeled E48 IgG. Note the high activity in the primary tumor of the tongue. On this image the lymph node metastases in the left subdigastric and midjugular levels of the neck are not visualized. (B) The left lateral image shows the primary tumor in the tongue. The lymph node metastases are not visualized. (C) Axial SPECT images show the primary tumor (arrow) as well as the subdigastric lymph node metastasis (arrowhead) in one axial plane and, separately (D), the midjugular lymph node metastasis (arrowhead) in a lower axial plane. A = anterior; P = posterior; L = left; R = right.

60% per side; the accuracy of RIS was 96% and 86% per level and 96% and 64% per side, respectively.

The capacity of RIS in the detection of tumor-involved neck levels/sides, which were missed (false-negative score) or detected (true-positive score) by palpation, CT and/or MRI, is shown in Table 3.

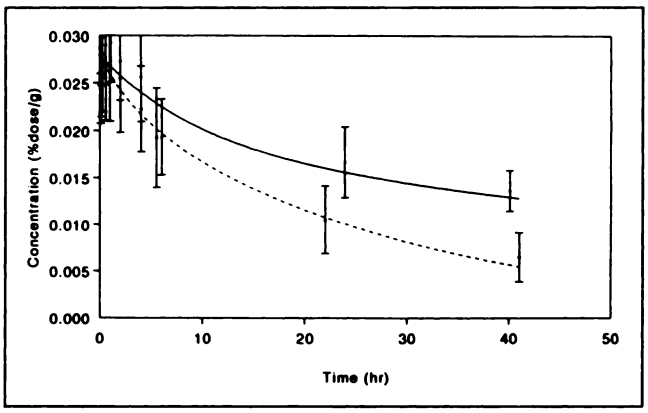

FIGURE 5. Average blood disappearance curves of ^{99m}Tc-labeled E48 F(ab')$_2$ (– –) and E48 IgG (——) from 15 and 17 patients, respectively.

Using the 95% confidence intervals of the diagnostic test, no significant differences in sensitivity, specificity and accuracy were found between RIS and the other diagnostic tests, except for RIS and palpation with respect to the sensitivity per side in the E48 IgG patient group. However, in a 99% confidence interval, no significant difference was found. The κ values per level for RIS with Mab E48 and palpation, CT and MRI per level in the whole group of patients were 0.682, 0.689 and 0.698, respectively. The κ values of the different diagnostic methods per level ranged in the E48 F(ab')$_2$ group from 0.622 to 1, whereas in the E48 IgG group values between 0.444 and 0.877 were found.

Of the 23 tumor-containing levels in the E48 F(ab')$_2$ group, 4 were missed while 11 out of 24 in the E48 IgG group were missed. The paraffin slides of the missed metastatic lymph nodes were reexamined histopathologically. The false-negative findings proved to be all lymph nodes containing small tumor deposits (ranging from micrometastasis to tumors of 9 × 9 mm) or tumor deposits containing a large proportion of necrosis (n = 2), keratin (n = 3) or fibrin (n = 5) deposits. In one patient, the missed node in Level IV was close to Level III. The node also contained a small amount of tumor. In another patient,

TABLE 1
Correlation of Preoperative Diagnostic Findings with Histopathological Findings for Each Level

	TP	FN	FP	TN	Sensitivity	Specificity	Accuracy	PPV	NPV
$F(ab')_2$ (n = 15)									
Palpation	15	8	4	90	65	96	90	79	92
CT	18	5	0	94	78	100	96	100	95
MRI	15	5	0	65	75	100	94	100	93
RIS	19	4	1	93	83	99	96	95	96
IgG (n = 17)									
Palpation	15	9	5	75	63	94	87	75	89
CT	14	10	9	71	58	89	82	61	88
MRI	11	11	4	73	50	95	85	73	87
RIS	13	11	4	76	54	95	86	76	87
All (n = 32)									
Palpation	30	17	9	165	64	95	88	79	91
CT	32	15	9	165	68	95	89	77	92
MRI	26	16	4	138	62	97	89	87	90
RIS	32	15	5	169	68	97	91	86	92

TP = true-positive; FN = false-negative; FP = false-positive; TN = true-negative; PPV = positive-predictive; NPV = negative-predictive value.

there was a high uptake of activity in the mouth, possibly obscuring activity uptake in a right submandibular lymph node metastasis. Two missed paralaryngeal lymph nodes were located too close to a larynx carcinoma. In one case, histopathological examination of a missed lymph node revealed metastases of an undifferentiated small cell tumor while the primary tumor showed locally squamous cell differentiation only.

Of the 94 tumor-free levels in the E48 $F(ab')_2$ group, 1 was falsely scored positive, while 4 out of 80 in the E48 IgG group were scored false-positive. For tumor-free sides, these figures are 0 out of 9 in the $F(ab')_2$ group and 2 out of 5 in the IgG group.

DISCUSSION

A number of animal and patient studies have shown that $F(ab')_2$ fragments are better suited for radioimmunoscintigraphic detection of tumors than whole IgG, due to the higher tumor-to-nontumor ratios obtained with $F(ab')_2$ fragments (*18,19,20*). Also for Mab E48 $F(ab')_2$, when tested in a HNSCC xenograft-bearing nude mice, we ob-

TABLE 2
Correlation of Preoperative Diagnostic Findings with Histopathological Findings

	TP	FN	FP	TN	Sensitivity	Specificity	Accuracy	PPV	NPV
$F(ab')_2$ (n = 15)									
Palpation	14	2	0	9	88	100	92	100	82
CT	15	1	0	9	94	100	96	100	90
MRI	12	1	0	5	92	100	94	100	83
RIS	15	1	0	9	94	100	96	100	90
IgG (n = 17)									
Palpation	15	2	3	2	88	40	77	83	50
CT	14	3	3	2	82	40	73	82	40
MRI	12	4	3	2	75	40	67	80	33
RIS	11	6	2	3	65	60	64	85	33
All (n = 32)									
Palpation	29	4	3	11	88	79	85	91	73
CT	29	4	3	11	88	79	85	91	73
MRI	24	5	3	7	83	70	79	89	58
RIS	26	7	2	12	79	86	81	93	63

TP = true-positive; FN = false-negative; FP = false-positive; TN = true-negative; PPV = positive-predictive value; NPV = negative-predictive value.

TABLE 3
Results of RIS in False-Negative and True-Positive Findings by Palpation, CT and MRI

	RIS (per level)		RIS (per side)	
	Seen	Missed	Seen	Missed
False-negative				
Palpation	6/17 (35%)	11/17 (65%)	2/4 (50%)	2/4 (50%)
CT	5/15 (33%)	10/15 (67%)	1/4 (25%)	3/4 (75%)
MRI	6/16 (38%)	10/16 (62%)	1/5 (20%)	4/5 (80%)
Palpation, CT and MRI	3/11 (27%)	8/11 (73%)	1/3 (33%)	2/3 (67%)
True-positive				
Palpation	26/30 (87%)	4/30 (13%)	26/29 (90%)	3/29 (10%)
CT	28/32 (87%)	4/32 (13%)	26/29 (90%)	3/29 (10%)
MRI	23/26 (88%)	3/26 (12%)	23/24 (96%)	1/24 (4%)
Palpation, CT and MRI	27/32 (84%)	5/32 (16%)	24/26 (92%)	2/26 (8%)

served such a superior quality for imaging (*10*). For this reason, initial RIS studies were performed with E48 $F(ab')_2$. We recently reported on the first 10 head and neck cancer patients imaged with ^{99m}Tc-labeled E48 $F(ab')_2$ and mentioned the high uptake of activity in the mouth which may hamper the diagnosis of submandibular or subdigastric lymph nodes. We hypothesized that oral activity may be less when using whole IgG, one of the reasons to modify the initial study and to also evaluate Mab E48 IgG for its value in RIS (*11*).

Considering the sensitivity and accuracy of data presented in this study for RIS with E48 IgG and $F(ab')_2$, it is tempting to state that $F(ab')_2$ fragments are better suited for clinical RIS than whole IgG. The sensitivity and accuracy per level was found to be higher for E48 $F(ab')_2$ when compared to E48 IgG, 83% and 96% versus 54% and 86%. However, one should be careful with the interpretation of these data since CT and MRI also scored better in the E48 $F(ab')_2$ group. Furthermore, the agreements (κ-values) between RIS and palpation, CT and MRI in the $F(ab')_2$ patient group were generally better as compared to those in the IgG patient group. This was also the case for the agreements (κ-values) between palpation, CT and MRI in these patient groups (data not shown). These data suggest differences in the composition of the groups. The fact that in the $F(ab')_2$ and the IgG patient groups, the sensitivity and accuracy for palpation, CT, MRI and RIS were found to be similar, indicates that $F(ab')_2$ and IgG are equally well suited for RIS. To obtain additional information, we recently started a new protocol in which biodistribution data are obtained from surgical specimens of patients who receive ^{131}I-labeled E48 IgG and ^{125}I-labeled E48 $F(ab')_2$ simultaneously.

In the present study the pattern of oral accumulation of activity is different for IgG when compared to $F(ab')_2$. In case of $F(ab')_2$, activity is apparently distributed throughout the whole oral cavity, but accumulation seems to be restricted to a smaller area with IgG (Figs. 2 and 3A). Due to its reduced activity uptake in the oral cavity, the IgG may be favourable for the detection of submandibular or subdigastric lymph nodes. However, from the present study, no conclusions can be drawn on this point due to the low numbers of submandibular and subdigastric lymph nodes. Definite proof requires sequential imaging procedures with IgG as well as with $F(ab')_2$ in patients with tumor involvement in these neck regions.

For the detection of lymph node metastases, the diagnostic value of RIS with Mab E48, either E48 $F(ab')_2$ or E48 IgG, is comparable to that of palpation, CT and MRI as shown by sensitivity, specificity and accuracy (Tables 1 and 2).

Until now, a limited number of clinical RIS studies in head and neck cancer patients have been described (*21–24*). Results obtained in these studies are difficult to compare. First, only a small proportion of the 5–15 patients included in these studies had lymph node involvement, thus limiting accurate calculation of sensitivity and specificity. Second, studies differ considerably with respect to (1) patient selection, (2) control of tumor deposits for antigen expression, (3) scintigraphic methods, (4) the way of topographical evaluation of the diagnostic findings and (5) the way to confirm scintigraphic findings. With respect to this latter difference, it is not clear whether the examiner for RIS was blinded to the results of the other diagnostic examinations.

A most important question in our study is: can RIS with Mab E48, either IgG or $F(ab')_2$, contribute to the preoperative staging of the neck in HNSCC patients, e.g., by complementing other methods used to assess nodal involvement? All patients who have nodal disease will need treatment of the regional lymphatics, while the neck needs no treatment if no metastasis is present. Recently the concept of selective neck dissection has been introduced (*25*). Selective neck dissections are used in patients with only minimal nodal disease with the intention to reduce morbidity. Another tendency is a "wait and see" policy for the clinically negative neck. Aforementioned treatment and policy are, however, only acceptable if the assessment of

the neck nodes is reliable. The assessment of the status of the neck in HNSCC patients, however, remains a problem. Not all enlarged nodes contain metastatic deposits. Nodes containing small deposits of carcinoma may not be enlarged. All current imaging techniques except US-guided aspiration cytology use morphological criteria, whereas RIS uses specific binding of the Mab to target cells.

In the present study, RIS, palpation, CT and MRI were directly compared for their value in diagnosis of lymph node metastases. For each diagnostic modality, findings were recorded per side as well as per lymph node level and compared with histopathological findings. In our experience, correlation of all image data per deposit, as performed in other studies, is inaccurate because precise localization of the deposits is impossible due to the lack of anatomical structures on the immunoscintigrams. It can be anticipated that the development of an accurate method to correlate all image modalities ("fused images") may resolve this problem. We think, however, that for the assessment of the potential of RIS for staging of the neck, analysis for tumor involvement per side as well as per level is satisfactory.

In one patient, RIS with Mab E48 was able to detect tumor-involved lymph nodes, which were not detectable by other examinations, thus contributing to the assessment of the status of the neck. In this patient receiving E48 $F(ab')_2$, RIS resulted in upstaging of three levels and one side. In this study, only 25% of the true-positive RIS levels were clearly visualized on planar images. Also, SPECT images provided most information in all other cases. The smallest tumor-involved lymph node detected with SPECT had diameters of 5 and 9 mm in the axial plane with a tumor load of more than 50%. From the clinical point of view it is important to consider the consequences when decisions for surgery in the present study would have been based on RIS only. In that case, 12 neck dissections (out of 14), which appeared to be free of tumor upon histopathological examination, would have been prevented by RIS. On the other hand, seven neck sides would have been undertreated while two sides would have been overtreated.

A major limitation of RIS with Mab E48 IgG or $F(ab')_2$ in its present form is the high percentage of false-negative scores. A total of 15 of 47 levels and 7 of 33 sides were scored false-negative. All missed tumor-involved lymph nodes were smaller than 2 cm in diameter. In these cases RIS was apparently unsuccessful due to the limited amount of antigen accessible for the Mab and possibly the limited sensitivity of a gamma camera. Consequently, micrometastases, small tumor-involved nodes, tumor-involved nodes with much keratin, and necrosis or fibrosis with only a small proportion of viable tumor cells were not diagnosed. Fibrosis may also hamper Mab penetration to tumor cells. Close spatial relation of a metastatic node to an area of increased uptake may also hamper diagnosis, i.e., para-
laryngeal nodes in larynx carcinoma and a submandibular node close to the mouth. While primary tumors were checked for antigen expression by immunohistochemistry on preoperative biopsies, we were not able to do so on the missed lymph node metastases. For proper histopathologic examination at our hospital, surgical specimens are routinely fixed in formalin and embedded in paraffin, resulting in a loss of E48 antigenicity.

Five levels and two sides were incorrectly scored positive. No clear explanation for these observations can be given. False-positive findings in RIS may be caused by blood-pool activity and asymmetric anatomy of the neck. Three of these levels were also incorrectly scored positive by palpation, CT and/or MRI. These findings indicate enlargement of the lymph nodes in these levels.

In conclusion, imaging data presented in this study indicate that RIS is as reliable as the other imaging techniques. Despite this, we believe that RIS with Mab E48 in its present form will not sufficiently contribute to a more reliable selection of patients who should be treated on behalf of their neck or patients in whom a "wait and see" policy is warranted. For this purpose the percentage of falsely scored negative neck levels per sides should be diminished. In this respect, the developing field of PET might result in a diagnostic modality with a higher sensitivity. Other disadvantages of RIS at the moment, limiting the routine application, are the complexity and high costs in comparison to CT and MRI. The selective accumulation of radioactivity in tumor tissue in combination with the known intrinsic radiosensitivity of HNSCC, however, justifies the development of radioimmunoconjugates for radioimmunotherapy (RIT). From this point of view RIS can be regarded as a prelude to RIT. Among other possibilities, RIT may be especially attractive in an adjuvant setting or in support to external beam irradiation. In our department, preparations for a Phase I adjuvant RIT trial with ^{186}Re-labeled chimeric Mab E48 IgG are in progress (*26,27*).

ACKNOWLEDGMENTS

The authors thank Drs. Hein Leverstein and Richard P. Wong Chung for clinical support; Henri Greuter for biochemical support; and Dr. Chris J.L.M. Meyer for histopathological support. This work was supported by the Dutch Ministry of Economic Affairs and by Centocor Europe, Inc., Leiden, the Netherlands.

REFERENCES

1. Snow GB, Annayas AA, Van Slooten EA, Bartelink H, Hart AAM. Prognostic factors of neck node metastases. *Clin Otolaryngol* 1982;7:182–192.
2. Leemans CR, Tiwari RM, Van der Waal I, Karim ABMF, Nauta JJP, Snow GB. The efficacy of comprehensive neck dissection with or without postoperative radiotherapy in nodal metastases of squamous cell carcinoma of the upper respiratory and digestive tracts. *Laryngoscope* 1990;100:1194–1198.
3. Leemans CR, Tiwari RM, Nauta JJP, Van der Waal I, Snow GB. Regional lymph node involvement and its significance in the development of distant metastases in head and neck cancer. *Cancer* 1993;71:452–456.
4. Ali S, Tiwari RM, Snow GB. False-positive and false-negative neck nodes. *Arch Otolaryngol Head Neck Surg* 1985;8:78–82.
5. Van den Brekel WM, Castelijns JA, Stel HV, Golding RP, Meijer CLJM, Snow GB. Modern imaging techniques and ultrasound guided aspiration cytology for the assessment of the neck node metastases: a prospective comparative study. *Eur Arch Otorhinolaryngol* 1993;250:11–17.

6. Goldenberg DM. Future role of radiolabeled monoclonal antibodies in oncological diagnosis and therapy. *Semin Nucl Med* 1989;19:332–339.
7. Quak JJ, Balm AJM, Van Dongen GAMS, et al. A 22 kD surface antigen detected by a monoclonal antibody E48 is exclusively expressed in stratified squamous and transitional epithelia. *Am J Pathol* 1990;33:191–195.
8. Quak JJ, Gerretsen M, Schrijvers AHGJ, Meijer CJLM, Van Dongen GAMS, Snow GB. Detection of squamous cell carcinoma xenografts in nude mice by radiolabeled monoclonal antibody E48. *Arch Otolaryngol Head Neck Surg* 1991;117:1287–1291.
9. Quak JJ, Balm AJM, Brakkee JPG, et al. Localization and imaging of radiolabeled monoclonal antibody E48 against squamous cell carcinoma of the head and neck in tumor-bearing nude mice. *Int J Cancer* 1989;44:534–538.
10. Gerretsen M, Quak JJ, Suh JS, et al. Superior localization and imaging of radiolabeled monoclonal antibody E48 $F(ab')_2$ fragments in xenografts of human squamous cell carcinoma of the head and neck and the vulva as compared to monoclonal antibody E48 IgG. *Br J Cancer* 1991;63:37–44.
11. Van Dongen GAMS, Leverstein H, Roos JC, et al. Radioimmunoscintigraphy of head and neck cancer using ^{99m}Tc-labeled monoclonal antibody E48 $F(ab')_2$. *Cancer Res* 1992;52:2569–2574.
12. Hermanek P, Sobin LH, eds. *TNM classification of malignant tumors, 4th edition*. Berlin: Springer Verlag; 1987:13–26.
13. Fritzberg AR, Abrams PG, Beaumier PL, et al. Specific and stable labeling of antibodies with technetium-99m with a diamide dithiolate chelating agent. *Proc Natl Acad Sci USA* 1988;85:4025–4029.
14. Van den Brekel MWM, Castelijns JA, Stel HV, et al. Detection and characterization of metastatic cervical adenopathy by MR imaging: comparison of different MR techniques. *J Comput Assist Tomogr* 1990;14:581–589.
15. Van den Brekel MWM, Stel HV, Castelijns JA, et al. Cervical lymph node metastases: assessment of radiologic criteria. *Radiology* 1990;177:379–384.
16. Shah JP, Strong E, Vikram B. Neck dissection: current status and future possibilities. *Clin Bulletin* 1981;11:25–33.
17. Fleiss JL. *Statistical methods for rates and proportions*. New York: John Wiley; 1981.
18. Wahl RL, Parker CW, Philpott GW. Improved radioimaging and tumor localization with monoclonal $F(ab')_2$. *J Nucl Med* 1983;24:316–325.
19. Buraggi GL, Callegaro L, Mariani G, et al. Imaging with ^{131}I-labeled monoclonal antibodies to a high-molecular-weight melanoma-associated antigen in patients with melanoma: efficacy of whole immunoglobulin and its $F(ab')_2$ fragments. *Cancer Res* 1985;45:3378–3387.
20. Goldenberg DM, Goldenberg H, Sharkey RM, et al. Imaging of colorectal carcinoma with radiolabeled antibodies. *Semin Nucl Med* 1989;19:262–281.
21. Tranter RMD, Fairweather DS, Bradwell AR, Dykes PW, Watson-James S, Chandler S. The detection of squamous cell tumours of the head and neck using radio-labelled antibodies. *J Laryngol Otol* 1984;98:71–74.
22. Soo KC, Ward M, Roberts KR, et al. Radioimmunoscintigraphy of squamous carcinomas of the head and neck. *Arch Otolaryngol Head Neck Surg* 1987;9:349–352.
23. Kairemo KJA, Hopsu EVM. Immunoscintigraphy in laryngeal and pharyngeal carcinomas using indium-111-labeled monoclonal antibody. *Acta Oncol* 1990;29:533–538.
24. Timon CI, McShane D, Hamilton D, Walsh MA. Head and neck cancer localization with indium labelled carcinoembryogenic antigen: a pilot project. *J Otolaryngol* 1991;20:283–287.
25. Byers RM, Wolf PF, Ballentyne AJ. Rationale for elective modified neck dissection. *Arch Otolaryngol Head Neck Surg* 1988;10:160–167.
26. Visser G, Gerretsen M, Herscheid J, Snow GB, Van Dongen GAMS. Labeling of monoclonal antibodies with ^{186}Re using the MAG_3 chelate for radioimmunotherapy of cancer: a technical protocol. *J Nucl Med* 1993;34:1953–1963.
27. Gerretsen M, Visser GWM, Van Walsum M, Meijer CJLM, Snow GB, Van Dongen GAMS. Rhenium-186-labeled monoclonal antibody E48 IgG mediated therapy of human head and neck squamous cell carcinoma xenografts. *Cancer Res* 1993;53:3524–3529.