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Immunoscintigraphy of Bone Sarcomas—Results in 5 Patients

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The feasibility of using the murine monoclonal antibody, TP-1, for clinical immunoscintigraphy was examined in a pilot study involving 5 patients with bone sarcomas. ^{131}I-labelled F(ab')$_2$ antibody fragments were injected in doses of 0.8–1.0 mg (90–130 MBq), and the accumulation of radioactivity was examined by scintigraphy, and assessed by direct measurements on biopsied tumour and normal tissue. One osteosarcoma patient had a primary tumour in the femur, whereas the other 4 had single lung metastases detected by other diagnostic methods. Immunoscintigraphy of the femoral primary was optimally visualised after 22 h. In 2 patients, the method failed to detect lung metastasis, in 1 of the cases possibly related to less than optimal methodological conditions. In 2 other patients, increased accumulation of radioactivity indicated one and three lung tumours, in addition to the single metastasis observed by X-ray and CT scanning, tumours that were later confirmed and removed surgically. The concentration of radioactivity in tumour and normal tissues 44–72 h after antibody injection could be measured in 4 patients. The tumour to blood ratios were in the range of 1.2–4.2, compared to 0.1–0.8 for various normal tissues. The results indicate that immunoscintigraphy with TP-1 antibody fragments have a potential for early detection of lung metastases in patients with bone sarcoma.

Key words: bone sarcomas, immunoscintigraphy, imaging, osteosarcoma, monoclonal antibody
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INTRODUCTION

IN SPITE of considerable therapeutic progress during the last two decades, more than one third of patients with osteogenic sarcomas succumb to the disease [1]. Micrometastases in the lungs are known to be present at an early stage. Thus, overt lung tumours developed in approximately 80% of the patients who, upon diagnosis, appeared to have localised disease, and were treated with amputation only [2, 3]. Moreover, in more than 80% of the patients that relapse after surgery and chemotherapy, the lungs are the first and only site of detectable metastatic spread and the ultimate cause of death [2, 4].

Importantly, osteosarcoma lung metastases frequently have a subpleural localisation, often in definite, limited numbers, allowing radical lung resections to be made, a treatment that may be curative [5, 6]. In fact, studies have shown that excision of solitary pulmonary metastases developing in patients previously treated with aggressive combination chemotherapy may cure as many as 30% of the cases [6, 7]. Therefore, early detection and localisation of such lesions are essential.

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Current methods for diagnosing lung metastases in osteosarcoma patients suffer from insufficient sensitivity and specificity. Results obtained in other types of cancer during the past decade have demonstrated that tumour metastases may be visualised by the use of radiolabelled monoclonal antibodies (MAbs), indicating that under appropriate conditions immunoscintigraphy may represent an attractive diagnostic modality [8, 9]. Here we report initial scintigraphic studies in patients with bone sarcomas using a murine anti-sarcoma MAb that previously has shown promising results in preclinical *in vitro* and *in vivo* studies [10–12].

PATIENTS AND METHODS

Patients

5 male patients, 4 with osteosarcoma and 1 with a clear cell chondrosarcoma (Table 1), were examined. Patient 1, who was imaged before the initial biopsy was taken, had a primary tumour in the distal left femur. The tumour was diagnosed radiologically, and subsequently histologically confirmed to be an osteosarcoma, but there was no evidence of lung metastasis. The other 4 patients had previously been successfully treated for primary bone sarcomas, and presented with single lung metastases detected by conventional methods. In these cases, selected for thoracic surgery, we had previously established by immunohistology that the TP-antibody bound to the primary tumour [12]. None of the patients were treated with chemotherapy or radiation during this study.

Table 1. Clinical parameters and results of immunoscintigraphy studies in 5 male patients with bone sarcoma

Patient number	Age	Site of primary tumour	Histological diagnosis	Injected dose: MAb (mg)	Injected dose: Radioactivity (MBq)	No. of tumours detected by: Immunoscintigraphy	No. of tumours detected by: Other non-surgical methods
1	19	Left distal femur	Osteogenic sarcoma (osteoblastic/sclerotic)	0.8	90	1	1
2	16	Right distal femur	Osteogenic sarcoma (osteoblastic)	0.9	120	0	1
3	22	Left proximal tibia	Osteogenic sarcoma (osteo-/chondroblastic)	0.9	100	0	1
4	56	Left proximal femur	Clear-cell chondrosarcoma	1.0	100	4	1
5	29	Left distal femur	Osteogenic sarcoma (osteoblastic)	0.8	130	2	1

Monoclonal antibody

The TP-1 MAb of subclass IgG2a was purified from mouse ascites by affinity chromatography on a protein-A-Sepharose column (Pharmacia, Uppsala, Sweden). This antibody recognises an epitope on an 80-kDa cell surface antigen seemingly associated, in a complex manner, with the bone isoenzyme of alkaline phosphatase [11, 12]. The IgG was eluted with 0.2 M acetate buffer (pH 4.7), immediately adjusted to pH 7 with solid Tris, vacuum concentrated using collodium bags (Schleicher and Schüell, Goettingen, Germany) and dialysed against phosphate-buffered saline (PBS), pH 7.3.

The $F(ab')_2$ fragments were prepared from IgG dissolved in 0.2 M acetate buffer (pH 4.2) by digestion for 6 h with pepsin (2:100, w:w) enzyme/substrate ratio. Undigested IgG and Fc fragments were removed by passing the reaction mixture through a Protein-A-Sepharose column from which the $F(ab')_2$ fragments were eluted unretarded with PBS, pH 7.3. Under these conditions, a yield of 45% was obtained. Possible lipopolysaccharides present in the preparation were removed by passage through a column containing 3 ml Detoxigel (Pierce, Rockford, U.S.A.) and elution with PBS, pH 7.3.

Radiolabelling procedure

The $F(ab')_2$ antibody fragments were labelled under sterile conditions with ^{131}I (IFE, Kjeller, Norway) by the Iodogen method [13], as described previously [11]. The labelling efficiency varied from 85 to 95% in the various preparations. The labelled immunoglobulin fraction was purified by gel filtration (Sephadex G-25). The amount of free iodide and iodate in the final product was less than 1%, as assessed by 10% trichloroacetic acid precipitation and electrophoresis. The specific activity in different preparations ranged from 100 to 160 MBq/mg. Labelled preparations were diluted in saline containing 1% human serum albumin (HSA) and sterilised by filtration through a 0.22 μm Millipore filter. Endotoxin levels in the injected material were less than 250 EU/20 ml, as measured by the Limulus-lysate test.

The immunoreactivity at antigen excess, of the injected radiolabelled preparations as well as in plasma samples obtained from the patients at various time points after the injection, were determined according to Lindmo and colleagues [14], using the antigen-positive osteosarcoma cell line OHS [15]. The immunoreactivity of the different injected preparations used in this study varied from 45 to 65%.

Scintigraphic imaging

The patients were imaged at different time points after the injection of the labelled MAb using a Siemens orbiter gamma camera with a ZLC 7500 camera head, equipped with a medium/high energy collimator (360 keV). The single photon emission computed tomography (SPECT) studies were performed with 60 views, each of 30 s duration, and the images were reconstructed in a Siemens ECT processor. A ramp filter was used, and the resulting images were smoothed in three dimensions using binominal weight factors, with four terms in the directions of the reconstruction planes and seven terms in the axial direction.

Biodistribution studies

Thyroid uptake of radionuclide was blocked by oral administration of 30 mg potassium iodide three times daily for 1 week, starting 24 h prior to the infusion of labelled MAb. Before imaging, all patients were exposed to an intradermal test dose of 1 μg of antibody fragments and observed for 3 h to detect possible skin hypersensitivity as indication of circulating pre-existing human anti-mouse IgE. Thereafter, approximately 1 mg of radiolabelled fragments (Table 1), diluted in 20 ml saline containing 1% HSA, was injected intravenously (i.v.) in the course of 10 min with careful monitoring of the patient.

Blood samples were collected at different time points after the injection of the radiolabelled MAb. The blood clearance of radioactivity was estimated by measuring the radioactivity in plasma samples obtained at different time points after the injection of the radiolabelled MAb.

Tumour material as well as samples from some normal tissues were obtained at surgery, performed within 44–72 h after injection. The content of radioactivity in blood and tissue samples was counted in a multi-well type gamma-counter (LKB, Bromma, Sweden), and expressed as cpm/g tissue and per cent of injected dose/kg tissue using a sample of the injected material for comparison.

RESULTS

Toxicity

None of the 5 patients showed a positive skin test. However, 2 h after the infusion, patient 5 experienced mild, flu-like symptoms and a transient temperature rise to 38.6°C that lasted for 4 h. Apart from this, no adverse effects were seen, and all the laboratory parameters remained within normal levels.

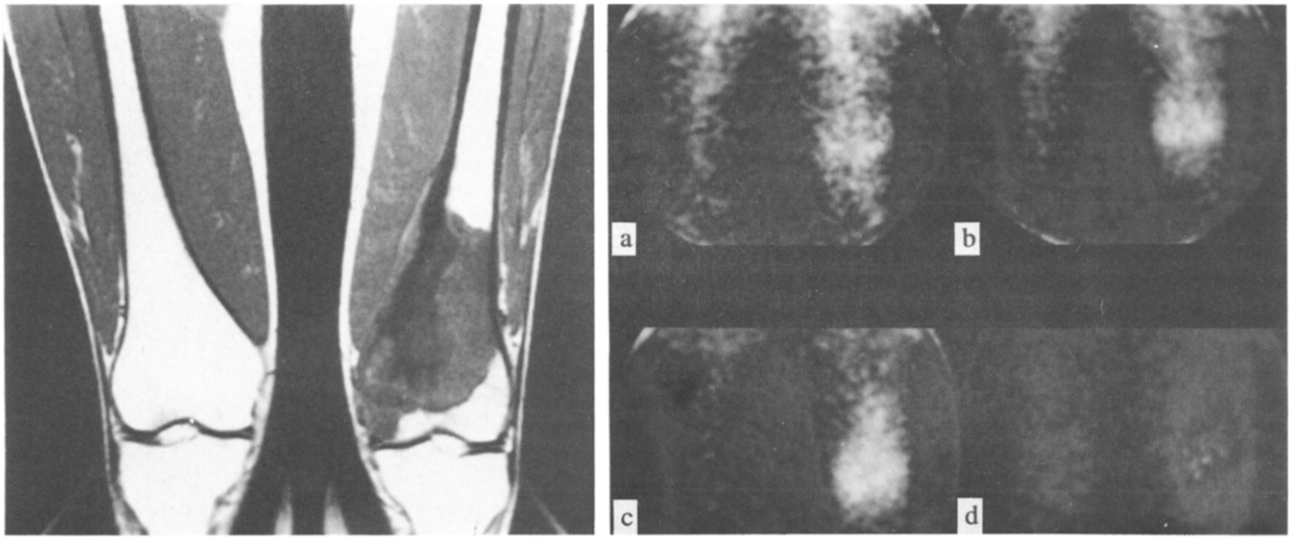

Figure 1. Immunoscintigraphy of patient 1 having a primary osteosarcoma in his left distal femur. Left: MRI scan. Right: immunoscintigraphy; planar images obtained at different time points (a:1, b:4, c:22 and d:52 h) after the injection of 0.8 mg TP-1 F(ab')$_2$ labelled with 90 MBq ^{131}I.

Blood clearance

The blood clearance of radioactivity showed, as expected, a biphasic profile (data not shown). The initial drop in blood content, which reflects the distribution to the organs, varied substantially between the different patients ($T_{1/2}\alpha$ 2.0 h ± 1.0 S.D.). In contrast, the second phase reflecting the renal excretion and degradation of the murine immunoglobulin fraction, showed a remarkably constant $T_{1/2}\beta$ (23.86 h ± 2.86 S.D.). Three hours postinjection, the radioactivity content in the blood had decreased to 50–30% of the value measured after 5 min (data not shown).

Since it is well known that murine MAbs injected into humans degrade rapidly, it was of interest to measure the fraction of the total blood radioactivity associated with the antibody. It was found that the immunoreactive fraction showed a gradual reduction with time, from about 40% of the injected radioactivity after 1 h down to about 5% at 72 h, although with significant differences between individual patients (data not shown).

Tumour targeting

The targeting potential of the ^{131}I-labelled TP-1 F(ab')$_2$ fragments was studied by whole body gamma-scintigraphy and

Table 2. Radioactivity in tissue samples obtained at surgery after injection of ^{131}I-labelled F(ab')$_2$ antibody fragments

Patient number	Time post injection (h)	Tissue	Radioactivity content (% of injected dose/kg)	Tissue-to-blood ratio
1	72	Tumour: centre	0.22	1.2
		periphery	0.31	1.4
		Muscle	0.11	0.5
		Fat	0.13	0.6
		Skin	0.12	0.6
2	60	Viable tumour	1.24	4.2
		Necrotic tumour	0.23	0.8
		Lymph node with tumour	0.44	1.1
		Muscle	0.11	0.4
		Fat	0.14	0.4
		Skin	0.22	0.8
		Lung	0.24	0.8
3	58	Tumour: total	0.32	1.3
		centre	0.22	0.9
		periphery	0.44	1.7
		Fat	0.04	0.1
		Lung	0.10	0.3
4	44	Tumour 1	0.95	1.9
		Tumour 2	0.80	1.7
		Lung	0.10	0.2
		Muscle	0.06	0.1
		Fat	0.08	0.1

by measuring the radioactivity content of tissues obtained at operation. The number of previously known tumour foci in the various patients is given in Table 1.

Figure 1 shows that the antibody bound to the primary osteosarcoma in the left femur of patient 1. Thus, images taken at different times after MAb injection demonstrate accumulation with time and retention of radioactivity at the tumour site, in contrast to the rapid clearance seen in the normal tissues and in the contralateral femur. The best image was obtained at 22 h. After this, there was a substantial loss of radioactivity from the tumour site, probably reflecting dehalogenation of the radioactivity from iodine-labelled antibodies. In agreement with the imaging results, tumour specimens obtained 72 h after injection of the MAb showed positive ratios of radioactivity compared to normal tissues, that is, tumour to muscle 2.8, tumour to fat 2.4, tumour to blood 1.4 (Table 2). In this patient, no evidence for the presence of lung metastases was found. Thus, concurrent SPECT imaging of the thoracic region showed no localised radioactivity uptake, and after follow-up for more than 3 years, there is no evidence of relapse.

Patient 2 had undergone a right-sided thoracotomy 3 months earlier, during which six small lung metastases had been removed. Later, a single, small metastatic lesion in the lower posterior part of the left lung was disclosed by CT scanning. Thoracotomy performed 60 h postinjection revealed a tumour measuring 8 mm in diameter. This metastasis was not detected on the planar immunoscintigrams, but it should be noted that SPECT was not performed because of lack of patient compliance. Upon removal, the tumour was found to consist of viable malignant tissue with a high radioactivity content. Thus, 60 h after injection the tumour to blood ratio was as high as 4.2 (Table 2), and the tumour to lung ratio was 5.3. In addition, two previously undetected tumours were also removed. Histological examination showed that one of the lesions contained necrotic tumour tissue (4 mm in diameter) with pronounced calcification and a low radioactivity level, whereas the other tumour consisted

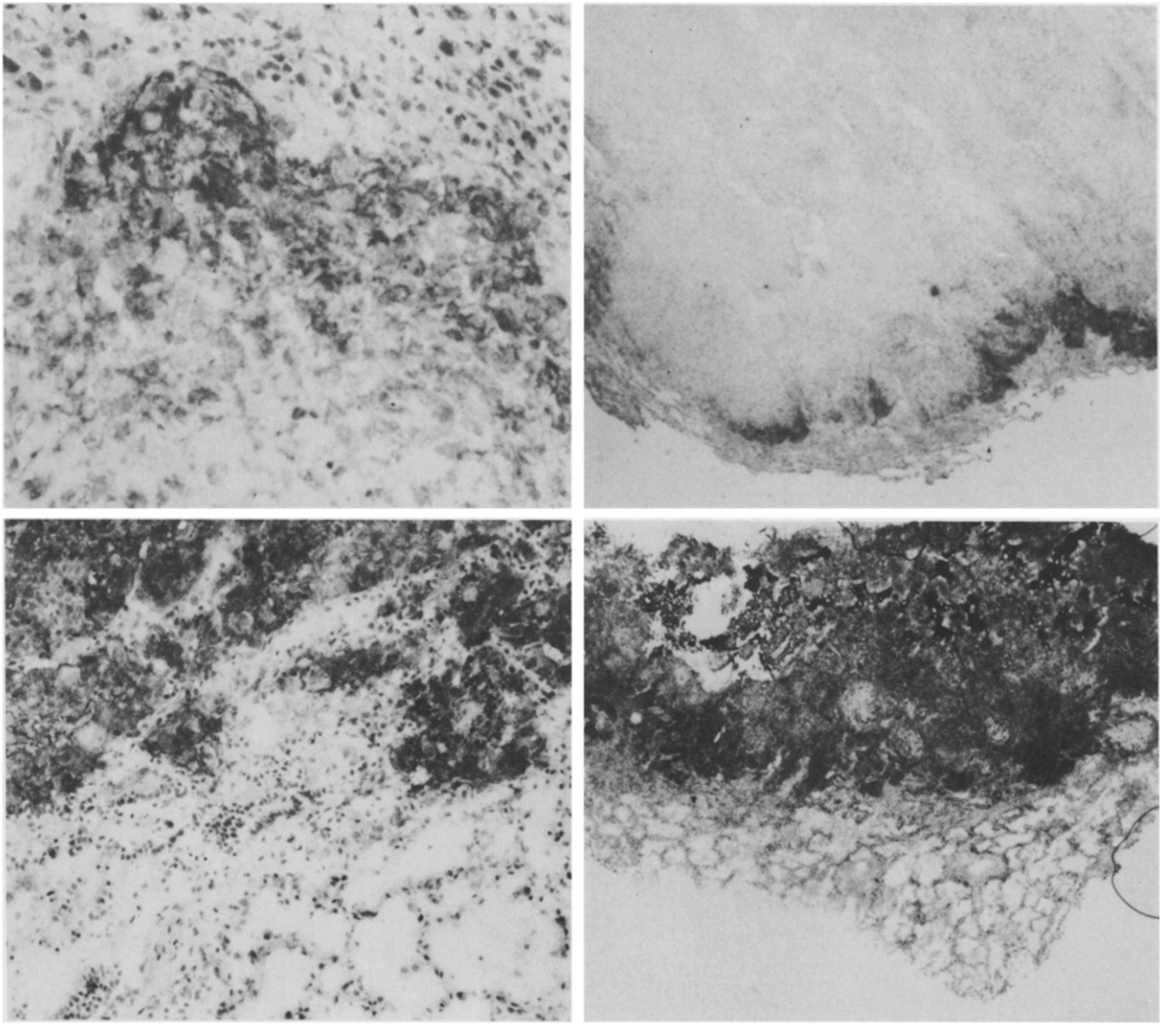

Figure 2. Immunohistological staining of frozen sections from lung metastases with the TP-1 MAb. Left panel: low-power magnification. Right panel: high-power magnification. Upper panel: homogenously-stained viable tumour tissue invading the lung (patient 2). Lower panel: positive staining of the thin rim of viable tumour tissue at the edge of the metastasis with unstained calcified necrotic centre (patient 3).

of a lymph node with a defined 3 mm osteosarcoma lesion with a somewhat higher amount of radioactivity (Table 2).

Patient 3 had a solitary lung metastasis disclosed by chest X-ray examination. The tumour was not seen on planar immunoscintigraphic images, and the SPECT data were suggestive of a metastasis, but were inconclusive. Upon removal of the lesion, histological examination showed heavily calcified necrotic tissue comprising at least 80% of the tumour volume (Figure 2), with a narrow rim of viable tumour cells in the periphery of the tumour. In agreement with this, the total lesion to blood radioactivity ratio was 1.3, with a value of 1.7 in the periphery of the tumour and 0.9 in the central region (Table 2).

Patient 4 had a slowly progressing clear cell chondrosarcoma originating in the proximal left femur. He had a metastasis (1.0 cm) in the left lung, visible on both chest X-ray and CT scanning. This metastasis was also found on SPECT, which, in addition, detected three other small areas with increased radioactivity uptake in the same lung. The interpretation of the SPECT images was, however, difficult because of a low signal-to-noise ratio. Upon surgery, it was found that the lung segment, comprising the previously-known lesion, also contained a separate metastasis (0.7 cm), clearly representing one of the areas with increased accumulation of radioactivity.

Another interesting finding was made in Patient 5. This patient had a solitary osteosarcoma metastasis in the lung which was not visible on chest X-ray but seen on CT scans. This metastasis was detected by SPECT immunoscintigraphy (Figure 3), in spite of its small size (1.0 × 0.8 × 0.7 cm measured after surgical removal). Importantly, an accumulation of radioactivity was also found laterally to this tumour (Figure 3). Although no palpable tumour corresponding to this location was found preoperatively, chest X-ray examination performed 9 months later identified a metastasis at this site, a finding confirmed upon reoperation. The patient is still in complete remission 2 years after the second thoracotomy.

DISCUSSION

Early detection of metastatic disease in osteosarcoma patients may significantly influence the choice of treatment. Standard diagnostic procedures employed in patients suspected to have metastatic disease include chest X-ray and radiological CT examination of the lungs and planar images obtained by bone scintigraphy using ^{99m}Tc-MDP. For the purpose of visualising larger metastases, these methods are simple and cost-effective, but the sensitivity is often inadequate for detection of small lung tumours. Whereas these diagnostic modalities are based on non-tumour-specific principles, radiolabelled antibodies with selective affinity for cell surface antigens present on osteosarcoma cells might, because of their targeting potential, be useful for visualising small tumour deposits.

Several MAbs recognising osteosarcoma-associated antigens [16–18] have been described. However, these have either shown unfortunate cross-reactivity with normal tissues, or inconsistent reactivity within each of the different sarcoma subtypes [19]. One clinical immunoscintigraphic study in osteosarcoma patients has so far been reported, in which the radiolabelled MAb 791T/36 was injected into 20 patients with known or suspected bone tumours [20, 21]. Although positive immunoscintigraphic images of the primary tumours were obtained in the 5 patients examined, all lung metastases diagnosed by other methods were missed.

The TP-1 MAb used here binds consistently, with high affinity, and almost exclusively to osteosarcoma cells [10, 12, 22, 23]. In the 5 patients studied here by immunoscintigraphy, both a primary and several metastatic lesions were visualised. The most interesting finding was the accumulation of ^{131}I-radiolabelled F(ab')$_2$ fragments in the areas of the lungs corresponding to foci of viable tumour cells. Moreover, only sarcoma tissue had radioactivity contents higher than that found in the blood. In one patient, a previously unknown metastasis was visualised at a time where no abnormality was detected by other methods, including manual exploration during thoracotomy. Importantly, 9 months later a single metastasis at this location was removed. To our knowledge, this is the first report where immunoscintigraphy has been successfully applied to detect subclinical lung metastases. Since surgical removal of lung metastases is potentially curative in this disease, such detection may have important clinical implications. This situation is exemplified by the fact that our patient is still disease-free 2 years after the second thoracotomy.

One limitation of immunoscintigraphy is the requirement for adequate blood supply to viable tumour tissue. The failure in

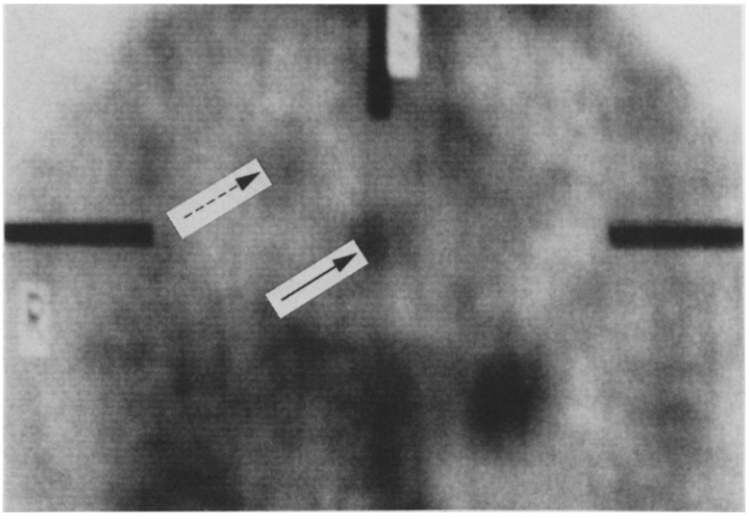

Figure 3. Immunoscintigraphic SPECT image of patient 5 with a known solitary metastasis in the right lung. Scan obtained 52 h after the injection of 0.8 mg ^{131}I-labelled TP-1F (ab')$_2$. Note blood pool activity in the liver, heart and the accumulation of free iodide in the stomach. → Known lesion. -→ Unknown lesion.

the prsent study to detect lung metastases present in 2 patients may partly be related to the finding that a major part of the tumour tissue in these cases was necrotic, and partly to the fact that SPECT could not be performed in 1 patient. However, compared to results obtained with other MAbs in bone sarcoma patients [20, 21], our data are quite promising. Moreover, since 4 of the 5 patients underwent thoracotomy 2–3 days after injection of the labelled antibody, the scintigraphy data could be further strengthened. Thus, it was possible to compare the results of the pre-operative examinations with the surgical findings, and to correlate the amount of radioactivity present in both tumour and normal tissues with the immunoscintigraphic images.

By optimising the choice of radionuclide and the coupling chemistry, as well as the dose of MAb administered, other investigators have succeeded in demonstrating the advantages of immunoscintigraphy compared to conventional diagnostic modalities in cancers such as neuroblastoma [24], melanoma [25], colorectal carcinoma [26] and ovarian carcinoma [27]. These findings suggest that it may be possible to substantially increase the resolution of the procedure employed here, and to develop it into a clinically useful, supplementary diagnostic method.

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